#### **ORIGINAL ARTICLE**

# Assessment of forage biomass and quality parameters of bermudagrass using proximal sensing of pasture canopy reflectance

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#### Keywords

Canopy reflectance; forage biomass; forage quality; grass pastures; model development and validation.

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Received 10 August 2006; accepted 10 November 2006

doi: 10.1111/j.1744-697X.2007.00072.x

#### **Abstract**

Assessments of forage productivity and quality during the growing season can help livestock managers make decisions for adjusting stocking rate and managing pastures. Traditional laboratory methods of forage quality determination are usually time consuming and costly. Remote sensing may provide a rapid and inexpensive means of estimating forage biomass and quality variables. Canopy reflectance measurements were made, using a spectroradiometer, in five warm season grass pastures during the 2002 and 2003 growing seasons to develop and validate algorithms to predict above-ground biomass, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) concentrations and CP availability (i.e. CP concentration × biomass yield) of the pastures. Forage biomass correlated  $(r^2 = 0.36, P < 0.0001)$  with a ratio of reflectance at 1145 and 1205 nm wavebands (R<sub>1145</sub>/R<sub>1205</sub>). Crude protein concentration and CP availability correlated linearly with  $R_{1695}/R_{605}$  and  $R_{875}/R_{735}$  ( $r^2 = 0.61$  and 0.47, P < 0.0001), respectively. Although NDF and ADF correlated significantly (P < 0.0001) with the reflectance ratios, the best reflectance ratios only explained 13-35% of ADF and NDF variations. Multiple regression (MAXR) models with a total of 10-waveband entrances improved the relationships between forage quality and canopy reflectance values ( $r^2 = 0.27 - 0.74$ , P < 0.0001). Validation of developed equations indicated that forage biomass, CP concentration, and CP availability could be predicted using either the reflectances at 10 wavebands or the two-band reflectance ratios. Pasture NDF could also be predicted using the 10-band MAXR equation ( $r^2 = 0.58$ ). Our results suggest that biomass and major quality parameters of warm season grass pastures can be rapidly and nondestructively predicted using canopy reflectance data.

#### Introduction

Pasture biomass productivity and quality values are crucial in management of grazing lands and livestock. More accurate and timely estimation of pasture biomass production and forage quality during the grazing season can help livestock managers make appropriate decisions of pasture fertilization and stocking rate. Traditional methods of laboratory chemical analysis have long been used for assessment of forage quality (Kellems and Church 1998). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) concentrations are commonly used forage quality variables (Ball *et al.* 

2001). These three quality variables are closely associated with intake potential, digestibility, and nutritive values of forage (Ball *et al.* 2001). Laboratory chemical methods used to determine these quality variables are time consuming and costly, and also require personnel with special skills. Additionally, the hazardous waste generated from laboratory processes must be disposed of in order to reduce the risk of environmental pollution.

Near infrared reflectance spectroscopy (NIRS) was developed, evaluated and used for determination of forage quality in the mid-1970s (Norris *et al.* 1976; Clark 1989). Since then, NIRS techniques have been increasingly used to quantify these forage

quality parameters (Moore *et al.* 1990). Compared with chemical procedures, NIRS analysis provides rapid and low-cost estimation of forage nutrient composition (Marten *et al.* 1989; Shenk and Westerhaus 1994). Although application of NIRS for determination of forage quality has great advantages compared to laboratory chemical methods, the NIRS analysis still requires a period of time for collecting, drying, and grinding vegetation samples.

Real-time measurements of reflectance at fresh leaf or canopy scale may offer an alternative for estimation of plant biochemical composition (Peñuelas and Filella 1998), especially leaf N and chlorophyll concentrations (Yoder and Pettigrew-Crosby 1995; Sims and Gamon 2002; Zhao et al. 2005). Serrano et al. (2002) developed two reflectance indices for assessments of vegetation nitrogen and lignin from AVIRIS data. Remotely sensed data at leaf, canopy or landscape level may be used to monitor plant physiology and biochemistry (Chappelle et al. 1992; Curran et al. 1992; Peñuelas and Filella 1998; Daughtry et al. 2000; Peñuelas and Inoue 2000; Serrano et al. 2002; Goel et al. 2003) and nutrient status (Read et al. 2002; Zhao et al. 2005), to detect environmental stresses (Gausman 1982; Chappelle et al. 1992; Filella et al. 1995; Blackmer et al. 1996; Ma et al. 1996; Voullot et al. 1998; Wang et al. 1998; Takahashi et al. 2000; Carter and Estep 2002), and to estimate yields of field crops (Ma et al. 1996; Plant et al. 2000; Reddy et al. 2003; Royo et al. 2003). However, similar studies on estimation of forage quality parameters (i.e. CP, NDF and ADF) using remote sensing are relatively limited. If the forage quality variables and biomass production can be predicted from nondestructive and timely measurements of canopy reflectance in a few wavebands via a spectroradiometer, it would further reduce laborious field sampling and sample processing procedures and would allow fine-scale mapping of the pasture productivity and nutritional status.

Changes in forage CP, NDF, ADF and above-ground biomass should affect canopy spectral reflectance values in a wide range of spectra 400-1695 nm, because plant canopy spectral reflectance is influenced by ground coverage, photosynthetic pigment contents, chemical composition and leaf structure (Campbell 1996; Peñuelas and Filella 1998; Kokaly and Clark 1999). Richardson et al. (1983) used a handheld radiometer to determine relationships between canopy reflectance values in red and near infrared wavebands and biomass or nitrogen (N) content of Alicia Bermuda grass and concluded that remote sensing would be useful for rangeland management. Everitt et al. (1985) investigated the relationship between leaf reflectance and leaf N concentration in buffelgrass and suggested that leaf reflectance at 500 and 550 nm highly correlated with leaf N concentration. More recently, Lamb et al. (2002) reported that leaf reflectance in chlorophyll red-edge (690-740 nm) could be used to estimate leaf N concentration and total N content of ryegrass.

Based on canopy reflectance measurements, concentrations of nitrogen, phosphorous, potassium, calcium and magnesium

in grass pastures can be predicated using continuum-removed absorption features (Mutanga et al. 2004). Studies have documented that forage biomass or N uptake closely correlates with the normalized difference vegetation index (NDVI) (Frank and Aase 1994; Frank and Karn 2003; Moges et al. 2004), but the relationships follow cubic (Frank and Karn 2003) or exponential models (Moges et al. 2004), rather than linear functions. It may be difficult to use a curvilinear or nonlinear model for prediction because of the asymptotic regions on the curves. Using modified partial least square regression methods, Starks et al. (2004) determined the relationships between pasture canopy reflectance in 252 narrow wavebands, covering the spectral region of 368-1100 nm, and forage NDF, ADF and N concentrations of bermudagrass (Cynodon dactylon, L.) pastures. They found that the forage quality parameters could be predicted using pasture canopy reflectance using the full wavelength range of the instrument. However, expensive hyperspectral radiometers are not practical for most livestock managers.

Starks et al. (2006) investigated correlations of forage NDF, ADF and CP concentrations with pasture canopy reflectance in broad wavebands of blue (450-520 nm), green (520-600 nm), red (R, 630-690 nm), near infrared (NIR, 760-900 nm), NIR/R and NDVI. They found that although the correlation coefficients of most measured forage quality variables with reflectances in all the broad wavebands or the reflectance indices (NIR/R and NDVI) are statistically significant, each could only explain a small portion of variability in the forage quality (i.e. small  $r^2$  values). Even though forage quality variables can be accurately estimated using hyperspectral remotely sensed data by integrating canopy reflectances in hundreds of narrow wavebands (Starks et al. 2004), the most important wavebands for forage quality estimation have yet to be determined and it is not clear whether a small subset of wavebands could be successfully used to predict the forage quality variables.

A 2-year experiment was conducted in five well-established pastures in central Oklahoma, USA for the purpose of selecting up to 10 wavebands for each quality variable in order to predict forage biomass, NDF, ADF and CP concentrations, and CP availability. The specific objectives of this study were to: (i) develop reflectance algorithms of each measured forage quality variable or above-ground biomass based on reflectance in a few selected wavebands or reflectance ratios; and (ii) validate the algorithms and evaluate model accuracy for real-time prediction of pasture nutritive values and biomass production.

## **Materials and methods**

#### **Experimental location**

The experiment was conducted at the USDA-ARS Grazinglands Research Laboratory (35°32′N, 98°02′W), El Reno, Oklahoma,

USA in the 2002 and 2003 growing seasons. Four monocultures of perennial warm season bermudagrass pastures of Midland, Ozarka,  $74 \times 12 - 12$ , and Common and one mixed pasture that consisted of approximately 70% Common and 30% other warm season grasses, were used for collection of biomass, forage quality and remotely sensed data. These grass cultivars are common in the Mid-South and South-East US livestock production areas. All the pastures were established in 1991 or 1992 with similar field size (3.2 ha), soil type of Brewer silt clay loam (fine-loamy, mixed, thermic Udic Rhodustalfs) and production management. Fields of the monocultural Common and the mixed pastures had a greater slope compared to the others. Midland, Ozarka,  $74 \times 12 - 12$  and the mixed pastures were grazed by beef cattle with a similar stocking rate (3 head ha<sup>-1</sup>), while the pasture of Common Bermuda grass was grazed by sheep (6 head ha<sup>-1</sup>) during the experiment. Fertilizer applications were based on soil test results and production recommendations for pasture management. Vegetation canopies of all the pastures completely covered the ground during data collection. Each field was split into eight plots for sampling. The plot size was approximately 0.4 ha.

#### Measurements

Canopy hyperspectral reflectances were measured on clear days between 10.00 and 12.30 hours (CST) from the eight plots in each pasture using a portable ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) during the growing seasons between June and September. The spectroradiometer measured canopy reflectance over the 350-2500 nm wavelength range and at a 1-nm sampling interval. The optical sensor of the spectroradiometer was mounted on a boom 2 m above and perpendicular to the soil surface. The radiometer had a 25° field of view (FOV), producing a view area with a diameter of 0.89 m. A Spectralon (Labsphere, Sutton, NH, USA) reference panel (white reference) was used to optimize the ASD instrument prior to taking three canopy reflectance measurements at each plot. The canopy reflectance data were expressed as relative values by dividing them by the white reference panel reflectance readings.

After the reflectance measurements were made in each sampling location, all vegetation in a 0.25-m² area within the ASD FOV was immediately clipped within 1 cm of the ground surface. Plant samples were transported to a laboratory and immediately dried in a forced air oven at 65°C for 72 h, weighed and then ground to determine the NDF, ADF and N concentrations in dry ground materials. Quantifications of forage NDF and ADF contents were based on the laboratory standard procedures of forage quality analysis outlined by Ankom Technology (Fairport, NY, USA). A detailed description of NDF and ADF determinations can be found at www.ankom.com/09\_procedures/procedures.shtml. Nitrogen concentration was determined using an automated combus-

tion instrument (Leco, St. Joseph, MI, USA). Forage CP concentration was calculated by multiplying the N concentration by 6.25 (Ball *et al.* 2001). Amounts of pasture CP availability (kg ha<sup>-1</sup>) were estimated by multiplying above-ground biomass by the CP concentration.

#### **Data analysis**

Reflectance values in three-wavelength ranges (i.e. 350–399, 1350-1449 and 1700-2500 nm) were first omitted from the reflectance datasets due to instrument noise or location of these wavebands within regions of atmospheric moisture absorption. The remaining reflectance data were averaged across 10-nm wavebands, giving a total of 120 narrow wavebands between 400 and 1700 nm. The spectral reflectance values measured on the three adjacent points in each plot at each sampling date were averaged. All the quality data and corresponding reflectance values were pooled over pasture fields, plots, sampling dates, and years (n = 414) in order to: (i) determine relationships between the pasture biomass or measured quality variables and canopy reflectance values or reflectance ratios at different narrow wavebands (10 nm); and (ii) develop reflectance algorithms for estimating pasture NDF, ADF and CP concentrations and biomass. The pooled data were randomly assigned into two equal-sized datasets, one for calibration and the other as a validation dataset.

Co-linearity or the codependence of various parameters is a major concern in multiple regression models, especially in hyperspectral remote sensing data analysis. Under such situations, the method of maximum  $r^2$  improvement (MAXR) is recommended (Yu 2000) and has been used for hyperspectral reflectance data analysis (Goel et al. 2003). We used several different regression methods to determine the best functional relationships between forage quality parameters and remotely sensed measurements of canopy reflectance. The three methods used in this study were: (i) simple linear regression (SAS 1997) of each forage quality variable with a selected two-band reflectance ratio (R<sub>band1</sub>/R<sub>band2</sub>) that has the greatest r<sup>2</sup> with the given quality parameter; (ii) multiple regression with the MAXR (SAS 1997) with a total of 10-waveband entrances; and (iii) partial least square regression with all the 120 wavebands (SAS 1997). Using the calibration dataset, all possible two-waveband reflectance ratios were first generated using SAS procedures (SAS 1997). Then simple correlation coefficients (r) of each forage quality variable with the reflectance ratios were determined. The reflectance ratios with the greatest |r| for each forage quality parameter were selected. Simple linear regression analyses were used to determine relationships between the individual quality variables and the selected reflectance ratios. The reflectance ratios or reflectance values recorded in various wavebands were considered as independent variables (x), and each of measured forage quality parameters was the dependent variable (y). The MAXR option,

under PROC REG in SAS statistical software (SAS 1997), was used to carry out multiple regression analyses of the data.

All forage quality parameters in the validation dataset were predicted based on the developed algorithms. Predicted biomass, NDF, ADF, CP and CP availability were plotted against the laboratory-measured results to evaluate performance of the algorithms. The root-mean-square error (RMSE) was calculated according to the following equation to determine the precision of estimation (prediction errors) between the measured values and predicted values:

$$RMSE = \sqrt{\left(\sum_{i=1}^{n} (\hat{y}_i - y_i)^2\right) / n}$$

where  $\hat{y}_i$  = the predicted values for a given quality variable,  $y_i$  = the true measured values, and n = the number of tested samples.

#### **Results and discussion**

#### Forage quality

Maximum, minimum, mean, standard deviation, and coefficient of variation (CV) for forage biomass and all measured quality variables across pastures, measuring dates, and plots in the 2002 and 2003 growing seasons are presented in Table 1. Averaged across pastures and sampling dates, above-ground biomass in 2002 and 2003 was 4.09 and 3.13 Mg ha<sup>-1</sup>, respectively; NDF, ADF and CP were 67, 33 and 9.4% of dry weight, respectively, in 2002 and 74, 34 and 8.3% of dry weight, respectively, in 2003. In 2002, CP availability was 383 kg ha<sup>-1</sup> while in 2003 it was 259 kg ha<sup>-1</sup>.

Among the five quality variables, NDF and ADF showed the smallest variation with CV of 4.9 and 2.7%, and 5.8 and 6.7%, respectively, in 2002 and 2003 (Table 1). Although the forage ADF concentration was comparable in the two growing seasons, the pastures in 2003 had a 10% higher NDF concentration, 12% lower CP concentration, 23% lower biomass and 32% lower CP availability, compared with the results in 2002. The differences in these forage quality variables between years could be associated with weather conditions, especially

**Table 1** Descriptive statistics of neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), above-ground biomass (BM), and CP availability of warm season grass pastures across genotypes and measuring dates in 2002 and 2003

		ADF	CP	Biomass	CP availability				
Parameter	NDF (%)	% of BM	% of BM	(Mg ha <sup>-1</sup> )	,				
2002 (n = 224)									
Maximum	75.3	38.6	19.44	8.97	996.7				
Minimum	57.2	26.4	4.84	1.04	64.8				
Mean	66.9	32.7	9.43	4.09	383.2				
SD	3.3	1.9	3.04	1.55	185.4				
CV (%)	4.9	5.8	32.17	37.9	48.4				
2003 (n = 19	0)								
Maximum	78.0	41.3	15.07	8.05	964.8				
Minimum	67.2	28.1	3.81	0.59	38.6				
Mean	73.9	33.7	8.32	3.13	259.4				
SD	2.0	2.3	2.37	1.44	143.9				
CV (%)	2.7	6.7	28.55	45.9	55.5				

SD = standard deviation; CV = coefficient of variation.

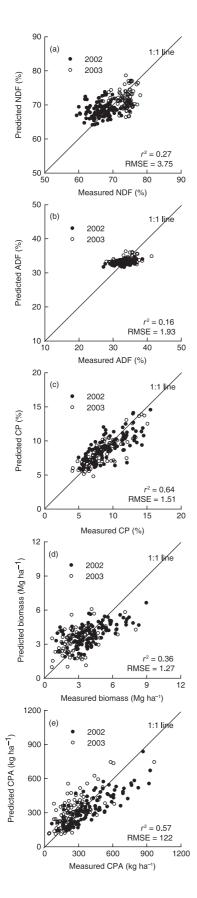
precipitation and air temperature (Starks *et al.* 2006), during plant growth and development. Total annual precipitation at the experimental location in 2003 (475 mm) was much lower than that in 2002 (794 mm). Less precipitation in the second growing season, accompanied with a higher air temperature, negatively affected pasture growth, biomass productivity and other forage quality parameters. The effects of precipitation and temperature on forage quality have been well summarized (Ball *et al.* 2001).

# Relationships between reflectance ratios and forage quality

The reflectance ratio most highly correlating with a forage quality variable (i.e. with the greatest  $r^2$ ) was selected and linear regression analysis was performed. The linear equations and  $r^2$  values between measured forage quality variables and respective reflectance ratios for all the quality parameters, derived from the calibration dataset, are presented in Table 2.

**Table 2** The best wavebands ( $\pm$  5 nm) selected from reflectance ratio calculation of the calibration dataset for determining of their linear relationships with forage NDF, ADF, CP, above-ground biomass (BM), and CP availability. The respective linear models and coefficients of determination ( $r^2$ ) are also presented (n = 207)

Quality parameter	Waveband	Equation	r <sup>2</sup>	
NDF (% of BM)	1695, 1645	$NDF = -263.83(R_{1695}/R_{1645}) + 330.72$	0.35	
ADF (% of BM)	1275, 1195	ADF = $60.93(R_{1275}/R_{1195}) - 32.11$	0.13	
CP (% of BM)	1695, 605	$CP = 2.075(R_{1695}/R_{605}) + 0.107$	0.61	
Biomass (Mg ha <sup>-1</sup> )	1145, 1205	Biomass = $21.474(R_{1145}/R_{1205}) - 19.599$	0.36	
CP availability (kg ha <sup>-1</sup> )	785, 735	CP availability = $1046.1(R_{785}/R_{735}) - 1050.9$	0.47	



Similar to previous reports in relationships between plant leaf N or chlorophyll concentration and reflectance measurements (Carter and Spiering 2002; Read et al. 2002; Zhao et al. 2005), using two-band reflectance ratios could improve the linear relationships between forage quality parameters and canopy reflectance measurements, as compared with the reflectance at single waveband (data not shown). Although the reflectance ratios  $R_{1695}/R_{1645}$ ,  $R_{1275}/R_{1195}$ ,  $R_{1695}/R_{605}$ ,  $R_{1145}/R_{1205}$ , and  $R_{785}/R_{735}$  had the largest linear correlation with NDF, ADF, CP, biomass and CP availability (P < 0.0001), respectively, these reflectance ratios could only explain 13-61% of variation of the forage quality variables. Of these forage quality variables, ADF had the poorest relationship with  $R_{1275}/R_{1195}$  ( $r^2 = 0.13$ ), while CP concentration had the strongest linear relationship with  $R_{1695}/R_{605}$  ( $r^2 = 0.61$ ).

Earlier studies on a number of plant species indicate that plant tissue N or CP concentration in dry, ground materials was most highly correlated with reflectance at wavelengths between 1200 and 2400 nm (Kokaly 2001). The absorbance peaks of protein or N are between 1020 and 2300 nm (Fourty *et al.* 1996; Serrano *et al.* 2002); however, in field settings moisture in plant tissue and in the atmosphere interferes with canopy reflectance in the wavelength ranges of 1350–1450 nm and 1800–1980 nm. Therefore, wavebands commonly used in estimation of N from dry, ground samples are less useful for forage N estimation from canopy reflectance measurements. The close relationships between forage CP concentration and live plant canopy reflectance ratio of R<sub>1695</sub>/R<sub>605</sub> in the present study may be helpful in avoidance of interference of moisture on plant tissue CP prediction.

#### Multiple regressions with MAXR

The best 1–10 wavebands for each forage quality variable and corresponding  $r^2$  values, model intercepts and partial regression coefficients are presented in Table 3. As expected, the MAXR-derived models showed an improvement with an increasing number of wavebands selected (Table 3). More specifically, the  $r^2$  values of NDF and ADF improved from 0.12 and 0.08, respectively, with the best one-waveband reflectance model to 0.72 and 0.27, respectively, when the best 10 wavebands were used. Crude protein concentration, above-ground biomass production, and CP availability were sufficiently associated with reflectances in the first five selected wavebands.

**Figure 1** Comparison of laboratory measured forage quality variables of (a) neutral detergent fiber (NDF), (b) acid detergent fiber (ADF), (c) crude protein (CP) concentration, (d) above-ground biomass, and (e) CP availability in the validation dataset (n = 207) with their predicted values based on the equations developed with the reflectance ratio in Table 2. The coefficients of determination ( $r^2$ ) and the root-mean-square error (RMSE) are presented.

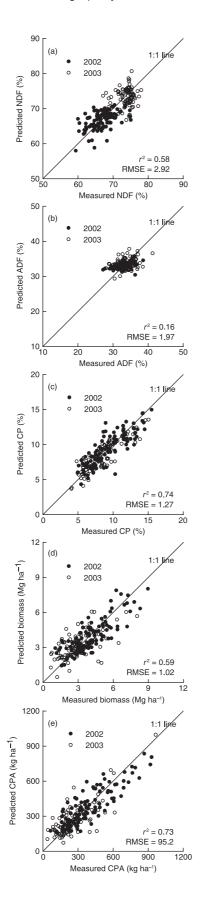
**Table 3** Useful wavebands for 1–10-reflectance variable regression models developed from the method of maximum  $r^2$  improvement (MAXR) for estimating NDF, ADF, CP, above-ground BM, and CP availability (CPA) of warm season grass pasture (n = 207). The  $r^2$  values, intercepts, and partial regression coefficients (the value in parenthesis) of the models are presented

Quality variable		Intercept	Selected waveband (nm)									
	r <sup>2</sup>		1	2	3	4	5	6	7	8	9	10
NDF (%)	0.115	81.6	1335 (–33.1)									
	0.174	71.0	1195 (-576.2)	1255 (541.3)								
	0.297	76.7	1175 (1831)	1185 (-2590)	1225 (720)							
	0.326	74.6	455 (119.5)	1175 (2265.3)	1185 (-2986)	1225 (676.9)						
	0.374	72.2	465 (159.2)	1175 (2362)	1185 (-1780)	1195 (-2803)	1215 (2170)					
	0.598	71.1	825 (115.3)	985 (-139.6)	1545 (-263)	1645 (2562)	1675 (-1263)	1685 (-1056)				
	0.612	69.9	795 (-348.7)	825 (530.8)	985 (-230.5)	1535 (-309)	1645 (2751)	1675 (–1251)	1685 (-1204)			
	0.627	69.9	795 (-349.3)	825 (513.4)	985 (-2084)	1565 (-824)	1605 (1017)	1645 (2119)	1675 (-1260)	1685 (-1055)		
	0.632	70.4	795 (-744.4)	825 (1084)	915 (-187.7)	985 (-200.1)	1555 (-642.1)	1605 (174.7)	1645 (2106)	1675 (-1235)	1685 (-1100)	
	0.720	71.0	635 (-5463)	645 (7496)	675 (-2141)	805 (-1192)	825 (1691)	895 (-450.6)	1155 (-326.7)	1165 (260.1)	1635 (1000)	1695 (-942.8)
ADF (%)	0.076	36.4	735 (-11.1)									
	0.114	35.2	605 (51.1)	715 (-31.2)								
	0.122	34.3	605 (74.7)	715 (-44.9)	835 (4.13)							
	0.187	35.0	1045 (-516.1)	1055 (532.2)	1155 (-35.3)	1475 (23.4)						
	0.209	34.5	1045 (-465.9)	1055 (480.5)	1225 (-230.8)	1245 (188.2)	1545 (27.4)					
	0.227	34.8	1045 (-582.6)	1055 (1025)	1065 (-427.4)	1225 (-242.2)	1245 (202.5)	1505 (27.6)				
	0.240	34.4	1045 (-567.9)	1055 (1023)	1065 (-437.9)	1215 (370.8)	1225 (-869.5)	1245 (451.6)	1545 (30.0)			
	0.249	33.9	905 (32.5)	1045 (-664.8)	1055 (1037)	1065 (-400.6)	1215 (400.2)	1225 (-910.4)	1245 (482.6)	1505 (28.3)		
	0.258	33.4	925 (41.0)	1045 (-583.3)	1055 (970.1)	1065 (-705.6)	1075 (289.5)	1215 (387.7)	1225 (-926.7)	1245 (498.5)	1545 (32.7)	
	0.269	32.9	925 (47.2)	1045 (-580.9)	1055 (943.7)	1065 (-736.1)	1075 (338.6)	1215 (435.4)	1225 (-1000)	1245 (522.3)	1495 (-258.7)	1505 (292.6)
CP (%)	0.376	14.2	605 (-86.5)									
	0.540	7.95	605 (-136.9)	1695 (38.6)								
	0.591	7.47	505 (311.7)	605 (-308.4)	1685 (34.2)							
	0.619	8.21	495 (326.0)	605 (-267.2)	1645 (-430.4)	1675 (449.3)						
	0.702	12.4	715 (-80.1)	1165 (-255.5)	1195 (715.0)	1285 (-712.8)	1295 (312.0)					
	0.706	11.9	445 (46.5)	715 (-87.0)	1165 (–225.9)	1195 (683.7)	1285 (-679.5)	1295 (280.5)				
	0.711	12.5	525 (171.3)	565 (-136.7)	715 (–76.2)	1165 (-242.3)	1195 (697.4)	1285 (-684.2)	1295 (285.4)			
	0.713	12.5	525 (173.9)	565 (-139.9)	715 (-74.9)	1165 (-250.5)	1195 (712.9)	1285 (-869.4)	1295 (600.9)	1305 (-138.1)		
	0.734	12.1	505 (192.9)	575 (-72.7)	715 (-81.8)	955 (-15.7)	1165 (–161.7)	1195 (614.6)	1285 (-384.7)	1615 (-232.8)	1685 (214.6)	
	0.738	11.9	505 (217.3)	565 (-91.7)	715 (-84.4)	745 (20.7)	935 (-25.5)	1165 (-143.7)	1195 (572.9)	1285 (-366.8)	1615 (-236.6)	1685 (219.9)

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Table 3 Continued

Quality variable <i>r</i>		Intercept	Selected waveband (nm)									
	r <sup>2</sup>		1	2	3	4	5	6	7	8	9	10
BM (Mg ha <sup>-1</sup> )	0.223	138.3	875 (–1667)									
	0.368	179.8	1145 (-4439)	1215 (1225)								
	0.475	82.8	1135 (-1280)	1215 (24204)	1285 (-11860)							
	0.543	118.9	1055 (-634)	1215 (-11501)	1315 (21272)	1485 (-10187)						
	0.565	137.7	1055 (-1661)	1205 (7015)	1265 (-21022)	1305 (25907)	1485 (-12024)					
	0.571	110.1	1055 (4751)	1205 (-3609)	1265 (11238)	1305 (-28002)	1465 (32284)	1485 (-15400)				
	0.576	121.9	1045 (9850)	1055 (-13586)	1205 (3797)	1265 (11093)	1305 (-27260)	1465 (32067)	1485 (-15724)			
	0.592	105.3	565 (15613)	725 (–24618)	1055 (15591)	1215 (-6976)	1265 (13242)	1305 (–31131)	1465 (33784)	1485 (-15646)		
	0.607	103.8	595 (20415)	685 (-31009)	735 (20136)	1005 (-9198)	1215 (13512)	1265 (-24407)	1305 (–25388)	1465 (55786)	1485 (-19163)	
	0.610	93.3	595 (7253)	485 (-72362)	735 (135415)	1005 (-67370)	1215 (–21567)	1265 (117418)	1305 (–282811)	1325 (92184)	1465 (121322)	1485 (–26733)
CPA (kg ha <sup>-1</sup> )	0.376	-174.6	785 (1282)									
	0.502	119.0	735 (–3625)	795 (3222)								
	0.522	61.3	735 (–5589)	755 (3798)	1145 (1256)							
	0.591	22.7	725 (–3693)	895 (5069)	1265 (-39160)	1295 (36929)						
	0.613	184.2	725 (-4126)	885 (4277)	1175 (10565)	1265 (-46096)	1295 (34588)					
	0.621	212.6	725 (–3915)	765 (1422)	1075 (4206)	1185 (–11541)	1265 (-50102)	1295 (35995)				
	0.626	223.0	725 (–4230)	765 (13630)	775 (–12899)	1055 (6520)	1195 (11572)	1265 (–57815)	1295 (42239)			
	0.632	222.9	725 (–4156)	765 (17367)	775 (–16803)	1075 (18616)	1095 (–12610)	1185 (11156)	1265 (–57505)	1295 (43003)		
	0.640	234.2	735 (-6010)	765 (19362)	785 (–17108)	1075 (17506)	1095 (–11323)	1185 (11102)	1265 (-58620)	1295 (44921)	1475 (-1144)	
	0.644	255.9	735 (–5889)	765 (18951)	785 (–16765)	1075 (17668)	1095 (-11708)	1185 (11313)	1265 (-56491)	1295 (42810)	1465 (6080)	1475 (-7187)



When wavebands were increased from one to five, the  $r^2$  values of CP concentration, biomass and CP availability were increased to 0.70, 0.57 and 0.61 from 0.37, 0.22 and 0.38, respectively (Table 3). When an additional five wavebands were entered into the models,  $r^2$  values changed little. These results indicated that each of the forage quality variables could be estimated using canopy reflectance in a few (5–10) wavebands.

#### Partial least square regression

Partial least square (PLS) regression can be a useful tool when there is no practical need to limit the number of measured factors in the prediction equation (Tobias 1995). Similar to the earlier report (Starks et al. 2004), the PLS models were highly significant for all the measured forage quality parameters in the present study (data not shown). The PLS regressions in our study only slightly improved model performance (i.e.  $r^2$ ) compared with multiple MAXR regression with 10-waveband entrances for all the measured forage quality variables. For instance, the PLS regression and cross-validation, reflecting model performances, of data in the calibration dataset indicated that the  $r^2$  between measured and predicted values of NDF, ADF, CP, biomass and CP availability were 0.52, 0.21, 0.75, 0.60 and 0.61, respectively. The RMSE of the cross-validation was 3.1% for NDF, 1.9% for ADF, 1.4% for CP, 1.05 Mg ha<sup>-1</sup> for biomass, and 125.3 kg ha<sup>-1</sup> for CP availability (data not shown). Results from the PLS regression further suggested that the variation of each forage quality variable mainly correlated with canopy reflectances in 5-10 wavebands rather than all the 120 wavebands.

# **Algorithm validation**

Each forage quality variable in the validation dataset was predicted using the equations in Table 2, based on the corresponding reflectance ratios. Scatter plots were used to further compare forage quality variables predicted by the respective equations of reflectance ratios with laboratory measurements (Figure 1). Among the five forage quality variables, CP concentration (Figure 1c) and CP availability (Figure 1e) could be well predicted using selected simple reflectance ratios with  $r^2$  values of 0.64 and 0.57 (n = 207, P < 0.0001), respectively. Although the RMSE between measured and predicted NDF and ADF were small (1.9–3.8%; Figure 1a,b), both of NDF and ADF were overestimated when laboratory measured values were

**Figure 2** Comparison of laboratory measured forage quality variables of (a) NDF, (b) ADF, (c) CP concentration, (d) above-ground biomass and (e) CP availability in the validation dataset (n = 207) with their predicted values based on the equations developed from the MAXR multiple regression of reflectances with the 10-waveband entrances. The coefficients of determination ( $r^2$ ) and the RMSE are presented.

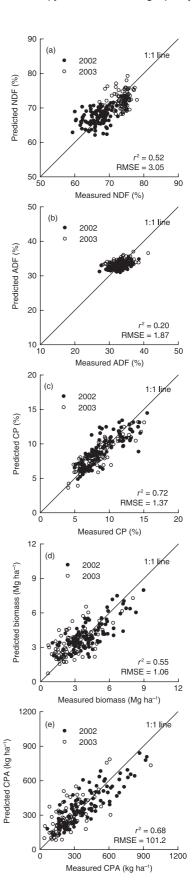
low and underestimated when measured values were high. Additionally, both had smaller  $r^2$  values (0.16–0.27) as compared with CP concentration or CP availability. Similarly, the relationship between measured and predicted above-ground biomass was relatively poor ( $r^2 = 0.36$ ) with a larger RMSE (Figure 1d).

Compared to simple linear models of reflectance ratios, the multiple regression models of MAXR with 10 wavebands considerably improved prediction accuracy of forage quality, except for ADF which had a small  $r^2$  value (Figure 2b). Low correlation of the canopy reflectance with ADF of pastures (Tables 2 and 3) and poor ADF prediction (Figures 1b and 2b) in the present study were probably associated with small variation in ADF (see Table 1). These results also indicated that it might be difficult to accurately predict bermudagrass forage ADF using canopy reflectance measurements even in 10 narrow wavebands.

Takahashi et al. (2000) examined various multivariate regression methods for prediction of rice crop dry weight and N accumulation using canopy hyperspectral reflectance and concluded that partial least square regression was the most useful among the models tested. In the present study, all PLS regression models developed from the calibration dataset were validated using data from the validation set to test the model efficiency and accuracy. Scatter plots of laboratory measured versus predicted for all the forage quality variables are presented in Figure 3. Compared to MAXR regression models with 10-waveband entrance in Figure 2, the PLS regression models incorporating all 120 wavebands did not improve prediction precision and accuracy of any forage quality variables (Figure 3). These results further indicate that when canopy reflectance is used to predict forage quality variables, reflectance at most wavebands contribute little to accurate prediction, and that a maximum of 10 wavebands for each quality variable is probably sufficient for the forage quality estimation.

In general, canopy reflectance depends not only on leaf morphological and biochemical characteristics of species (Daughtry and Walthall 1998; Mutanga *et al.* 2004), but also on the degree of vegetation canopy closure because exposed soils directly affect canopy reflectance features (Huete *et al.* 1985; Otterman *et al.* 1995). It should be noted that, in the present study, vegetation canopies of all the pastures were closed. Thus, any influence due to soil reflectance was minimized. Therefore, when extending our findings to other forage species or to more open canopies of grass pastures, soil effects on plant canopy reflectance must be taken into account.

**Figure 3** Comparison of laboratory measured forage quality variables of (a) NDF, (b) ADF, (c) CP concentration, (d) above-ground biomass and (e) CP availability in the validation dataset (n = 207) with their predicted values based on the equations developed from partial least square regression of reflectances with an involvement of all the 120 wavebands. The coefficients of determination ( $r^2$ ) and the RMSE are presented.



## **Conclusions**

The results of this study demonstrate the potential of using canopy reflectance data to estimate forage quality variables of warm season grass pastures. We compared several different methods of data analysis for prediction of the forage quality using canopy reflectance measurements. Simple reflectance ratios in specific narrow wavebands may be useful for timely prediction of live pasture CP concentration, above-ground biomass or CP availability. However, the relationship between the reflectance ratios and NDF or ADF content was poor. Compared to partial least square regression with all the 120 wavebands, MAXR multiple regression incorporating 5-10 wavebands produced similar coefficients of determination  $(r^2)$  and RMSE for prediction of the pasture biomass production and quality parameters (such as CP and NDF concentrations and CP availability). The ADF of the pastures had poor correlation with canopy reflectance data in all analysis methods used in this study due to a small range of variation. These results indicate that hyperspectral remote sensed data obtained from canopy or from landscape may offer a fast and inexpensive method for timely and nondestructive prediction of some major forage quality parameters.

# **Acknowledgments**

Appreciation is expressed for the excellent technical assistance provided by Dale Purdue, John Ross and Houston Cantrell in data collection and processing. We thank Drs W.R. Raun and W.B. Henry for internal review of the manuscript and for their helpful comments.

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